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Original Research Article

TERPENOIDS PROFILE OF *CLITORIA TERNATEA* LINN

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Abstract:
The present investigation was aimed to reveal the terpenoid profile of *Clitoria ternatea* Linn seed, stem and leaves using HPTLC. Preliminary phytochemical screening was carried out by Harborne method. HPTLC studies were followed by Harborne and Wagner et al. method. The n-hexane-ethyl acetate (7.2:2.9) was employed as mobile phase for terpenoids. The methanolic extracts of *Clitoria ternatea* stem, leaves and seeds showed the presence of 23 different types of terpenoids with 17 different Rf values with range 0.01 to 0.92. In general more degree of terpenoids diversity has been observed in vegetative parts when compared to the reproductive part (Seed). Maximum number [9] of terpenoids has been observed in leaves followed by seed (8). The proposed HPTLC profile can be used for the identification of the medicinally important plants and distinguish from its adulterant.
Keywords: HPTLC; Terpenoids; *Clitoria ternatea*; Phytochemistry

Introduction:

Nature depends on complex system of biosynthetic pathways to synthesise a group of small organic molecules essential to sustain life. All organisms naturally produce some terpenoids as part of primary metabolism, but many produce terpenoids via secondary metabolites.Terpenoids (also called "isoprenoids") represent one of the largest families of natural products includes more than 40,000 individual compounds of both primary and secondary metabolisms. Plants have mammoth ability to synthesize huge amounts of various terpenoids and subsequently utilized by humans for their precious applications. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. The biopotentials of terpenoid glycosides is well recognized, many have been found in plants used in traditional medicine[1-3]. Various terpenoids are contained in many

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plants for not only herbal medicine use but also dietary use [4]. As a result of medicinal potential and advancement in separation and analytical techniques, the rate of discovery of new terpenoids has increased over the last ten years. But there is no report on the repenoids profile of *Clitoria ternatea* L.

In pharmacognosy, the phytochemical assessment is one of the important and vital tool for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques such as fluorescence, UV-VIS, FT-IR, HPLC, HPTLC and GC-MS. The chromatographic technniques are exercised to distinguish the medicinal sources from its adulterants, consistency of plant products and accepted approach for detection and evaluation of the quality of plant medicines [5, 6]. HPLC and HPTLC both come out as efficient tool for the phytochemical assessment and generally accepted system for its high accuracy, precision and reproducibility of results. Of which, HPTLC has many advantages because of high sample throughput at low operating cost, easy sample preparation, short analysis time and analytical assurance [7,8]. Chromatographic methods play an vital role in the pharmaceutical field, hence, need to authenticate the method when they are developed and intended to be for everyday use. Kumar *et al* [9] have been developed a simple, precise, sensitive and selective protocol for the determination of taraxerol in *Clitoria ternatea* L. But there is no report on the HPTLC terpenoid profile of *Clitoria ternatea* seed, stem and leaves. With this background the present study was aimed to reveal the alkaloid profile of *Clitoria ternatea* Linn seed, stem and leaves using HPTLC.

Materials and Methods

Clitoria ternatea Linn was collected from natural habitats, Coimbatore District, Tamil Nadu, India, and authenticated by Dr. E.G. Wesely. The fresh materials of *Clitoria ternatea* stem, leaves and seeds were separated shade dried and powdered using the electric homogenizer. The powdered samples (25g) were extracted with 150 mL of methanol for 8 h by using the Soxhlet apparatus. Preliminary phytochemical screening was done by following the standard method described by Harborne [10], HPTLC studies were carried out following Wagner et al[11]. The n-hexane-ethyl acetate (7.2: 2.9) was employed as mobile phase for terpenoids. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with Anisaldehyde sulphuric acid reagent as spray reagent and dried at 120°C in hot air oven for 10 min. The plate was photo-documented at UV 366 nm and daylight using Photo-documentation (CAMAG REPROSTAR 3) chamber. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR3) and captured the images under White light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

Results and Discussion

The results of the preliminary phytochemical studies confirm the presence of terpenoids in the methanolic extracts of *Clitoria ternatea* stem, leaves and seeds. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using n-hexane-ethyl acetate (7.2 : 2.9) as the mobile phase [Table 1 - 4; Fig.1.A-J]. The methanolic extract of stem, leaves and seeds of *Clitoria ternatea* showed the presence of 23 different types of terpenoids with 17 different Rf values with range 0.01 to 0.92 [Table – 1 to 4]. In general more degree of terpenoids diversity has been observed in vegetative parts when compared to the reproductive part (Seed). Maximum number [9] of terpenoids has been observed in leaves followed by seed (8). Among the nine different terpenoids of leaves, six terpenoids with Rf values 0.11, 0.2, 0.26, 0.45, 0.82 and 0.88 are unique to leaves only [Table -1 & 4]. Similar to that Eight different types of terpenoids have been observed in seeds of *Clitoria ternatea*. The terpenoids with Rf values 0.01, 0.14, 0.5, 0. 54, and 0.64 are unique to the seeds and they are not present in other vegetative parts of the plant. The terpenoids with Rf values 0.47 and 0.70 are showed their unique presence only in the stems of *Clitoria ternatea*. The terpenoids with the Rf value 0.74 and 0.92 are displayed their comon expression in all the studied parts of *Clitoria ternatea*. The terpenoids with Rf value 0.62 demonstrated its presence only in the vegetative parts of *Clitoria ternatea*.

The HPTLC chromatogram developed using n-hexane: ethyl acetate solvent system showed the presence of 23 peaks with maximum area under the curve indicating the possible quantity of terpenoids in the methanolic extracts of root, stem, leaves, flower and seeds of *C. Clitorea* (Table 2). It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures the concentration and ratio of components in the parts of the plant.

Plant antioxidants are made of a mixture of different substances like ascorbic acid and tocopherols, polyphenolic compounds, or terpenoids. Plant antioxidants perform numerous important functions in plants and humans [12]. Additionally, their antioxidative capacity is believed to be responsible for the health promoting properties of fruits and vegetables. Currently, there is an increased interest in natural substances with valuable medicinal properties, such as terpenoids (hydrocarbon composition) and multiple C_5H_8. Plant terpenoids are employed widely for their aromatic qualities. Interestingly, effective ingredients in several plant-derived medicinal extracts are also terpenoid compounds of monoterpenoid, sesquiterpenoid, diterpenoid, triterpenoid and carotenoid groups. Inflammatory diseases and cancer are typical therapeutic indications of traditional medicines[13]. They play a vital role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Basic research about terpenoids by various methods, including chromatography, was carried out in the early 60's late 70's of the last century [14]. A comprehensive review about terpenoids, their sources, structures, uses can be found [15].

Table 1: HPTLC Terpenoids Profile of *Clitoria ternatea* methanolic extract

Rf	Seed	Stem	Leaves	Solanesol
0.01	+			
0.04	+	+		
0.11			+	
0.14	+			
0.20			+	
0.26			+	
0.45			+	
0.47		+		
0.51	+			
0.54	+			
0.62		+	+	
0.64	+			
0.70		+		
0.74	+	+	+	+
0.82			+	
0.88			+	
0.92	+	+	+	

Table 2: HPTLC Terpenoids Profile of methanolic extract *Clitoria ternatea* Seed

Rf	**Height**	**Area**	**Assigned substance**
0.01	235.2	2073.6	Unknown
0.04	26.6	232.5	Terpenoid 1
0.14	20.0	507.2	Unknown
0.51	16.3	441.1	Unknown
0.54	21.3	631.1	Unknown
0.64	35.3	1318.1	Terpenoid 2
0.74	63.9	2083.9	Terpenoid 3
0.92	140.0	8127.8	Terpenoid 4
0.74	400.9	10819.7	Solanesol standard

Table 3: HPTLC Terpenoids Profile of methanolic extract *Clitoria ternatea* Stem

Rf	Height	Area	Assigned substance
0.04	16.4	128.7	Terpenoid 1
0.47	31.6	1352.2	Terpenoid 2
0.62	70.4	1895.7	Terpenoid 3
0.70	23.0	595.9	Unknown
0.74	39.0	1292.7	Terpenoid 4
0.92	124.7	6362.5	Terpenoid 5
0.74	400.9	10819.7	Solanesol standard

Table 4: HPTLC Terpenoids Profile of methanolic extract *Clitoria ternatea* Leaves

Rf	Height	Area	Assigned substance
0.11	27.1	614.0	Terpenoid 1
0.20	39.9	1256.5	Unknown
0.26	36.4	1392.2	Unknown
0.45	66.3	3640.1	Terpenoid 2
0.62	104.1	3986.1	Terpenoid 3
0.74	75.9	2717.1	Terpenoid 4
0.82	56.9	1983.9	Terpenoid 5
0.88	106.5	3279.1	Terpenoid 6
0.92	147.9	5365.2	Terpenoid 7
0.74	400.9	10819.7	Solanesol standard

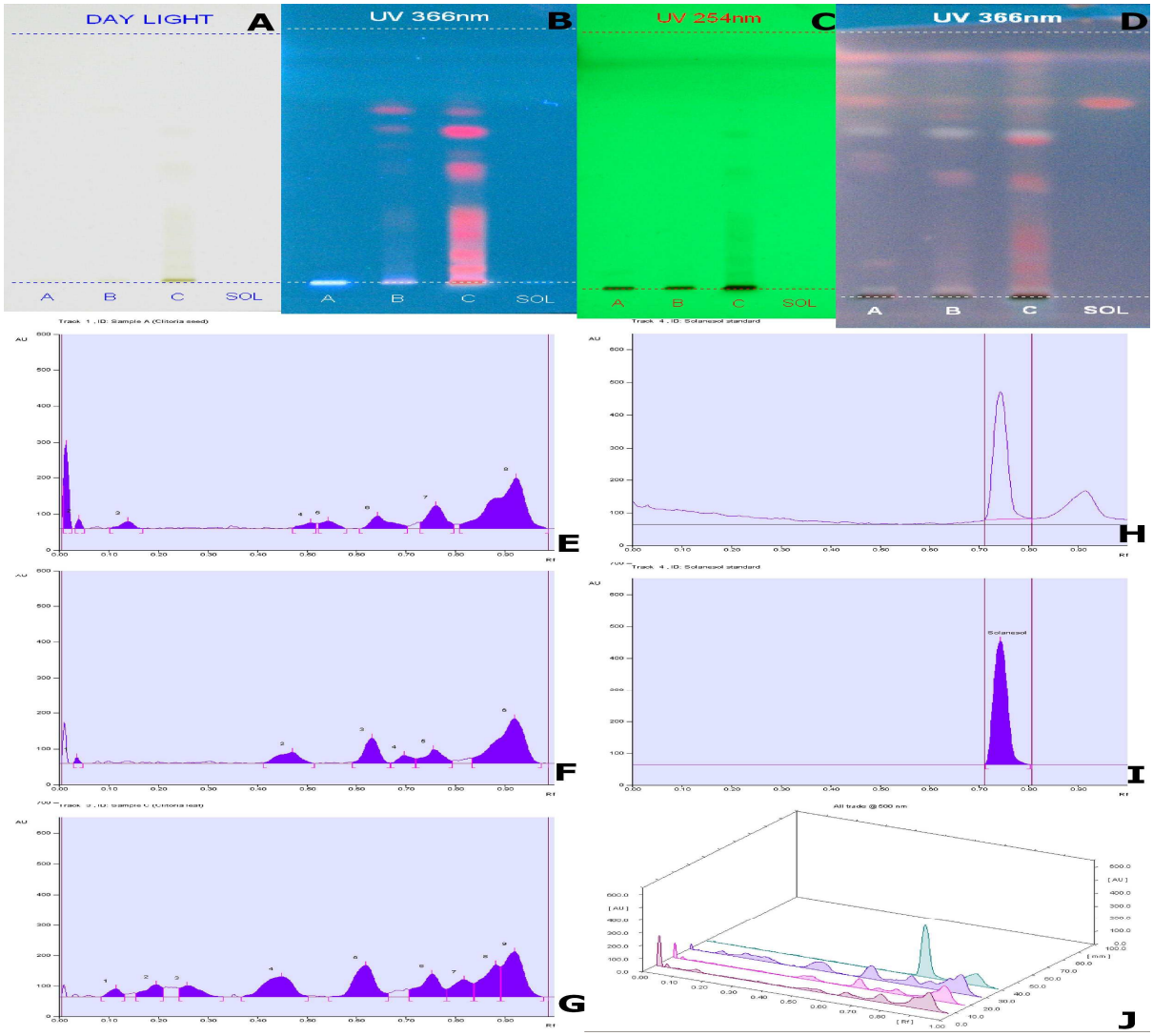

Fig. 1. HPTLC Terpenoids Profile and Chromatogram of *Clitoria ternatea* L.

A. HPTLC **Terpenoids** profile of the ***Clitoria ternatea*** under Day light
B. HPTLC **Terpenoids** profile of the ***Clitoria ternatea*** under UV 366
C. HPTLC **Terpenoids** profile of the ***Clitoria ternatea*** under UV 254
D. HPTLC **Terpenoids** profile of the ***Clitoria ternatea*** under UV 366 – After Derivation
E. HPTLC Chromatogram of methanolic extracts of ***Clitoria ternatea*** seed - Peak densitogram display - Scanned at 366 nm
F. HPTLC Chromatogram of methanolic extracts of ***Clitoria ternatea*** stem - Peak densitogram display - Scanned at 366 nm
G. HPTLC Chromatogram of methanolic extracts of ***Clitoria ternatea*** leaves - Peak densitogram display - Scanned at 366 nm
H. HPTLC Chromatogram of standard Solanesol Baseline display - Scanned at 500 nm
I. HPTLC Chromatogram of Standard Solanesol Peak densitogram display - Scanned at 500 nm
J. 3D display of HPTLC Chromatogram of ***Clitoria ternatea*** – seed, stem and leaves methanolic extracts

Chromatographic profile of metabolites can be used for the evaluation of quality uniformity and stability of herbal extracts or products by visible observation and comparison of the standardized profile [16]. In the present study we established the HPTLC terpenoid profile for the vegetative and reproductive parts of *C. ternatea* to identify and distinguish the *Clitoria ternatea* from the other crude drugs and its adulterants. The mobile phase n-hexane-ethyl acetate (7.2: 2.9) displayed the terpenoid presence in the *C.ternatea* seed with the Rf value 0.04, 0.64, 0.74 & 0.94 in stems with the Rf value 0.04, 0.47, 0.62, 0.74 & 0.92 and in leaves with the Rf value 0.11, 0.45, 0.62, 0.74, 0.82, 0.88 & 0.92. Of which, the band with Rf value 0.74 is matched with the standard Solanesol. The results of present study confirmed the terpenoid occurrence in the vegetative and reproductive parts and supported the multiple pharmaceutical applications of *C. ternatea*. The HPTLC method developed for the identification of *C. ternatea* is simple, precise, specific, accurate, rapid and cost effective. Developed HPTLC chromatogram of *C. ternatea* methanolic extracts of vegetative and reproductive parts could be used efficiently for identification, and quality assessment of the plant.

Conclusion

The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such chemoprofiles are useful in distinguish the medicinal source from its adulterant and the HPTLC profile can be used as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies. For developing analytical method pure active chemical constituents should be isolated in further study and identification on the basis of reference standard shall be made. This profile helps in setting in house standards of the medicinal plants used extensively by herbal manufactures.

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